Effect of photoreactivation on ultraviolet inactivation of *Microcystis aeruginosa*

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**ABSTRACT**

*Microcystis aeruginosa* forms algal bloom in lakes. They produce toxic compounds such as microcystin. Against such algal problems, the effect of UV treatment was examined. In UV treatment, the effect of photoreactivation should be examined. Photoreactivation is a repair mechanism of genomic DNA damage by sunlight irradiation. UV treatment causes DNA damages on target cyanobacteria, however sunlight can repair some of these DNA damages. To examine the effect of photoreactivation, both white and yellow light incubations were employed. White light allows both photoreactivation and photosynthesis, while yellow light prohibits photoreactivation and only allows photosynthesis. *Microcystis aeruginosa* NIES 98 strain and PCC 7806 strain were used as the test cultures. Those cultures were exposed to low-pressure (LP) or medium-pressure (MP) ultraviolet (UV) lamp, then incubated under white or yellow light. Yellow light incubation method was effective to examine photoreactivation. It was revealed that almost six times UV fluence was required to inactivate 99% of *Microcystis aeruginosa*, under photoreactivation condition, compared with non-photoreactivation condition. Inhibition of photoreactivation could greatly enhance UV treatment efficiency against *Microcystis aeruginosa*. One of the practical suggestions is to conduct UV treatment at night, when photoreactivation by sunlight rarely takes place. Highly efficient inactivation was achieved by avoiding photoreactivation.

**Key words** | *Microcystis aeruginosa*, photoreactivation, UV treatment

**INTRODUCTION**

The presence of algae in drinking water source can have a significant impact on the subsequent water treatment. Algae produce undesirable odorous compounds such as 2-methylisoborneol (2-MIB) or Geosmin (Chang *et al.* 1985; Hargensheimer & Watson 1996; Sugiura *et al.* 1998) and also produce toxic compounds such as microcystin (Carmichael 1992; Lambert *et al.* 1994; Humphage & Falconer 1999). Therefore, control of algae in the sources of drinking water has received considerable attention. The most effective and drastic way to control algal growth is to reduce nutrient loads into lakes or reservoirs (Ryding & Rast 1989). However, because of the significant internal loading in most reservoirs and lakes, especially from the bottom sediment, controlling the external nutrient-load alone is not sufficient to prevent seasonal algal blooms (Horne & Goldman 1994).

In order to inhibit the excessive algal growth and to reduce its impact on water treatment, many water treatment plants apply copper sulfate to their target lakes or reservoirs. Currently, however, there is a growing concern against the use of copper sulfate, mainly because it also has an impact on non-target creatures other than algae. Meanwhile, some water treatment utilities apply chlorine in order to inhibit the growth of algae, but chlorine reacts with the precursors of disinfection by-products in water to produce cancer-causing by-products such as trihalomethane (*Hoehn et al.* 1980).

Compared with the use of chemical compounds, UV treatment has certain advantages to improve the defects of chemical treatments. UV exposure leaves no chemical residuals in its germicidal effects and therefore has a less impact on the ecosystem in the target watersheds. Another advantage

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is that UV treatment gives relatively lower disinfection byproducts (Oppenheimer et al. 1997; Alam et al. 2001), because UV reacts with the target DNA much more efficiently than chemical agents in water. With these advantageous characteristics UV treatment is expected to become an alternative to conventional treatment against excessive algal growth.

As for the system of UV irradiation, most conventional UV lamps are low pressure UV lamps (LPUV) while medium pressure UV lamps (MPUV) have also been used. In order to apply UV to inhibit the excessive algal growth, attention should be paid to photoreactivation. In the photoreactivation process, UV-induced DNA damage can be repaired by the activity of photolyase with the energy of light whose wavelength is about 300 nm to 400 nm. Once microorganisms are exposed to UV light and their DNAs are damaged, those organisms can not reproduce themselves and therefore the growth is inhibited. However, when UV-damaged DNA is repaired by the photoreactivation process, the microorganisms can reproduce themselves normally. Hence, it can be easily presumed that the photoreactivation process may impair the efficacy of UV treatment. This study examined the effects of photoreactivation by employing the yellow light incubation method. Yellow light incubation allows photosynthesis but inhibits photoreactivation, because yellow light does not emit light between 300 nm to 400 nm.

In this study, the scope of using UV-radiation to control algal growth was assessed using M. aeruginosa as the test species. M. aeruginosa was selected for the experiment because of its frequent association with seasonal algal blooms. M. aeruginosa PCC 7806 and NIES-98 strain was selected as the representative Microcystis aeruginosa. The specific objectives were (i) to study the effect of UV-radiation on the inactivation of algae; and (ii) to study the level of photoreactivation by comparing two incubating conditions.

MATERIALS AND METHODS

Cultures

Two axenic cultures of the planktonic blue-green algae were used in this study. Microcystis aeruginosa (PCC 7806) was obtained from the Pasteur Culture Collection (Institute Pasteur, Paris, France), and M. aeruginosa (NIES-98) was obtained from National Institute for Environmental Studies (Tsukuba, Japan). M. aeruginosa (PCC 7806) was grown in a BG-11 medium (Sigma) and M. aeruginosa (NIES-98) was grown in an M-12 medium, according to the recommendations by those culture collections. Cultures were maintained at 25 C in an incubation chamber (BITEC-400L, Shimadzu) under controlled lighting. White light fluorescent lamps (FL20SW-B, GE/Hitachi) and yellow light fluorescent lamps (FL20SY-F, National) were used as the light sources with an automated light/dark cycle of 12 h/12 h. Light intensity during the lighting phase was 1500 lux. The white light fluorescent lamp emits wavelength range from 350 nm to 700 nm, while the yellow light lamp emits 450 nm to 700 nm. The wavelength range from 500 nm to 400 nm is cut out in the yellow light lamp, thus inhibits photoreactivation.

UV irradiation and subsequent incubation

Axenic cultures of M. aeruginosa were grown in a BG-11 or M-12 medium to reach an concentration of 10^5 or 10^6 cells ml^-1. To assess the effects of UV irradiation, 40 ml of each cultured algal suspension was irradiated in a glass petri dish with a 90-mm diameter and 0.45 cm depth. The BG-11 or M-12 medium was used as an exposure medium as well as the subsequent incubation media for pure culture. Absorbance of each media at 254 nm was 0.011 for M-12 medium and 0.09 for BG-11 medium. We used a monochromatic low-pressure UV lamp (15 W × 2, GE/Hitachi) and a polychromatic medium-pressure UV lamp (330 W × 1, B410MW, Ebara). The germicidal intensity of the light emitted from each lamp was standardized by determining the irradiance of light with a biodosimeter using an F-specific RNA coliphage Qβ (Kamiko & Ohgaki 1989). After irradiation, samples were incubated for 14 days in an incubation chamber (25 C, 1500 lux of fluorescent light, 12 h/12 h light/dark cycle) in 100-ml Erlenmeyer flasks. Experiments were conducted three times from UV irradiation to subsequent incubation and analysis and results are the mean of those three experiments. Error bars in the figures show the maximum or minimum of each data set.

Counting cell number

The number of cells in samples was determined using a fluorescence microscope (BH2, Olympus) with a plankton-counting chamber (MPC-200, Matsunami Glass, Japan). The numbers of cells were calculated immediately before and after UV irradiation, as well as 1, 3, 6, 10, and 14 days (24±2 h) of incubation after UV irradiation for M. aeruginosa PCC 7806 strain. For M. aeruginosa NIES-98 strain, cell number was counted immediately before and after UV irradiation, 1, 3, 5, 7 days after UV irradiation. Counted cell numbers were expressed as cell density in the figures.
Calculation of growth inhibitory effect

In order to calculate the growth inhibitory effect, the cephalosporin method (Sakai et al. 2009) was employed for *M. aeruginosa* PCC 7806 strain. This method was employed because growing cells and non-growing cells coexist in the flask after exposed to UV and those numbers need to be counted separately. Briefly, cell wall synthesis inhibitor cephalosporin was added into two flasks, that were exposed to same fluence of UV. Growing cells are destroyed under the addition of cephalosporin, and not destroyed without cephalosporin. By calculating the difference of the cell number of two flasks, number of growing cells was calculated. Then, the number of non-growing cells was calculated and shown as growth inhibitory effect by UV.

RESULTS

Cell number profile of *M. aeruginosa* after UV irradiation

Cell number profile of *M. aeruginosa* NIES-98 strain is shown in Figure 1 and *M. aeruginosa* PCC 7806 strain is shown in Figures 2 and 3. NIES-98 strain was incubated for 7 days, while PCC 7806 strain was for 14 days. Figure 1 shows the result of white light incubation (with photoreactivation), while Figure 1b shows the result of yellow light incubation (without photoreactivation). Under the photoreactivation condition, cell number of the control sample continued to increase within 7 days of incubation. 180 mJ cm\(^{-2}\) of MP UV sample also increased cell number. 180 mJ cm\(^{-2}\) of LP UV sample showed almost constant cell number profile at around \(10^5\) cells ml\(^{-1}\); growth was inhibited at 180 mJ cm\(^{-2}\) of LP UV. 600 mJ cm\(^{-2}\) of MP or LP UV samples decreased their cell number gradually from \(10^5\) cells ml\(^{-1}\) at day 0 to \(10^4\) cells at day 7. Cell number of 1800 mJ cm\(^{-2}\) of MP or LP UV samples dropped under the detection limit of 400 cells ml\(^{-1}\) at day 1. Figure 1b shows the result of yellow light incubation samples. Control sample increased their cell number for 7 days of incubation; difference of experimental condition did not affect the experimental result. 1800 mJ cm\(^{-2}\) of LP or MP UV was effective to decrease cell number within 2 days. 180 mJ cm\(^{-2}\) of LP or MP UV samples also decreased their cell number within 3 days, earlier than 600 mJ cm\(^{-2}\) samples. 600 mJ cm\(^{-2}\) of LP or MP UV samples decreased their cell number gradually to \(10^3\) cells ml\(^{-1}\) within 7 days. Cell number reduction rate was slower in 600 mJ cm\(^{-2}\) samples, even though UV fluence was larger than 180 mJ cm\(^{-2}\) samples.

Figure 2a shows the result of white light incubation (with photoreactivation), exposed to LP UV. Cell number of the control samples showed an increase until day 10, then slightly decreased. Under the photoreactivation condition, 30 and 60 mJ cm\(^{-2}\) of LP UV samples showed no clear growth inhibitory effect; cell number continued to increase throughout 14 days of incubation period. Cell number remained almost constant in 90, 120, 180 mJ cm\(^{-2}\) samples until 6 to 10 days. 600 or 1800 mJ cm\(^{-2}\) of LP UV samples showed cell number reduction until day 6. Cell number turned to increase after that. Therefore, it was clarified that *M. aeruginosa* PCC 7806 growth was inhibited at over 90 mJ cm\(^{-2}\).

Figure 2b shows the result of yellow light incubation (without photoreactivation), exposed to LP UV. Cell number of the control sample increased up to day 10, then slightly decreased. At 10 mJ cm\(^{-2}\) of LP UV samples, cell number did not increase for about 6 to 10 days. Cell number was constant or decreased until 6 days, when LP UV fluences were above 10 mJ cm\(^{-2}\). *M. aeruginosa* growth can be inhibited only by 10 mJ cm\(^{-2}\) of UV under non-photoreactivation condition.

Figure 3a showed the result of white light incubation (with photoreactivation), while Figure 3b showed the result of yellow light incubation (without photoreactivation), exposed to MP UV. There were no major differences between LP UV results in Figure 2 and MP UV results in Figure 3. It turned out that growth inhibitory effect was almost same both by LP UV or MP UV; *M. aeruginosa* PCC 7806 growth was inhibited at over 90 mJ cm\(^{-2}\) under photoreactivation condition, 10 mJ cm\(^{-2}\) under non-photoreactivation condition.

Growth inhibitory effect of UV on *M. aeruginosa* PCC 7806 strain

Effect of UV inactivation on *M. aeruginosa* PCC 7806 was calculated by the cephalosporin method (Sakai et al. 2009) and shown in Figure 4 as a log survival ratio. These calculation results will show more accurate growth inhibitory effect, compared with the cell number profile.

Log survival ratio of low pressure UV (LP UV) samples under white light incubation (LP-W) was almost zero at 0 and 30 mJ cm\(^{-2}\). It suddenly dropped to −1.0 log at 90 mJ cm\(^{-2}\), and gradually went down to about −2.0 log at around 180 mJ cm\(^{-2}\). Finally, it went down to −3.0 log or −4.0 log at 600 or 1800 mJ cm\(^{-2}\). In the case of MP UV-irradiated samples for white light incubation, general profile was almost the same as that of LP UV-irradiated samples. Almost same growth...
inhibitory effect was observed for white light incubation samples between LP UV (LP-W) and MP UV (MP-W).

Under yellow light incubation, log survival ratio of LP UV irradiated samples (LP-Y) suddenly dropped to around $-2.0$ log only at 30 mJ cm$^{-2}$. It gradually decreased at over 30 mJ cm$^{-2}$ from $-2.0$ log to $-4.0$ log. In the case of MP UV irradiated samples, profile was almost the same as that of LP UV samples.

There were no clear differences between LP and MP UV both for white or yellow light incubation. On the contrary,
there was a large difference between white and yellow light incubation, indicating a large effect by photoreactivation. 2 log inactivation was achieved by 30 mJ cm\(^{-2}\) of UV under non-photoreactivation condition and 180 mJ cm\(^{-2}\) of UV under photoreactivation condition.

**Cell number reduction rate of UV-irradiated *M. aeruginosa***

Cell number reduction rate was calculated, in order to clearly show the inconsistency between UV fluence and cell number reduction rate under non-photoreactivation condition. Those rates were calculated from the cell number difference at day 0 and day 7 for NIES 98 strain, and difference at day 0 and day 6 for PCC 7806 strain. When the cell number went down under the detection limit of 400 cells ml\(^{-1}\), cell number was calculated at the first day of under detection limit instead of day 7 or day 6. Under white light incubation, cell number reduction rate was increased as UV fluence was increased. However, the result was different in yellow light incubation samples. For NIES 98 strain, the rate was 0.85 and 0.87 for 180 mJ cm\(^{-2}\) of LP and MP UV irradiation, and 0.32 and 0.38 for 600 mJ cm\(^{-2}\) of LP and MP UV irradiation, respectively. It was higher in 180 mJ cm\(^{-2}\) samples than in 600 mJ cm\(^{-2}\) samples. For PCC 7806 strain as well, the rate was 0.12 and 0.09 for 30 mJ cm\(^{-2}\) of LP and MP UV irradiation, respectively, and 0.01 both for 120 mJ cm\(^{-2}\) of LP and MP UV irradiation. It was also higher in 30 mJ cm\(^{-2}\) samples than in 120 mJ cm\(^{-2}\) samples.

**DISCUSSION**

Role of photoreactivation was investigated in growth inhibition of *M. aeruginosa* by UV. In order to study the role of photoreactivation, white light incubation result and yellow light incubation result were compared. Two points were compared; growth inhibitory effect measured by cephalosporin and cell number reduction rate.

Growth inhibitory effect measured by cephalosporin was summarized in Figure 4. There was a great difference between white and yellow light incubation, both by LP or MP UV lamp. Two log inactivation was achieved by 180 mJ cm\(^{-2}\) under white light (with photoreactivation), while same inactivation was achieved only by 30 mJ cm\(^{-2}\) under yellow light (without photoreactivation). It was suggested that enough amount of DNA damage for 2 log inactivation was produced by 30 mJ cm\(^{-2}\) of UV, however, most of those damages were recovered under photoreactivation condition. Therefore, it could be a following task to inhibit photoreactivation aimed for more efficient inactivation of *M. aeruginosa*.

Growth inhibitory effect in Figure 4 was not significantly different over 180 mJ cm\(^{-2}\), regardless of photoreactivation. This also suggests the inactivation mechanism of *M. aeruginosa* over 180 mJ cm\(^{-2}\) was not so much related to DNA damage. Previous research suggests DNA damage itself can be repaired even at 1800 mJ cm\(^{-2}\) (Sakai et al., 2007). Therefore, possible damage might not be on molecular DNA but on cell metabolism, such as cell membrane or photosynthesis apparatus. It would also become a future task to pursue the cause of inactivation of *M. aeruginosa* at over 180 mJ cm\(^{-2}\) of UV.

Cell number reduction rate was another interesting point to discuss for yellow light incubation samples. In white light incubation samples, the rate was increased as UV fluence was increased. However, under yellow light incubation, it did not increase as UV fluence was increased. This phenomenon was observed in common both by LP or MP UV irradiation. For NIES 98 strain, the rate was higher in 180 mJ cm\(^{-2}\) samples than in 600 mJ cm\(^{-2}\) samples. For PCC 7806 strain, the rate was also higher in 20 and 30 mJ cm\(^{-2}\) samples than in 120 and 180 mJ cm\(^{-2}\) samples. The cause of the higher inactivation rate might be attributed to the relatively higher metabolic activity in 180 mJ cm\(^{-2}\) of NIES 98 strain samples and 20 or 30 mJ cm\(^{-2}\) of PCC 7806 strain samples. It could be
speculated those samples recognized DNA damages faster than other samples by the higher metabolic activity. That is, in samples received a higher UV fluence, Microcystis cells took more time to replicate themselves due to a larger damage on metabolic activity, which resulted in slower reduction of cell number. Reversely, in samples received a lower UV fluence, cells took less time to replicate themselves because of a smaller damage on metabolic activity, which resulted in a faster reduction of cell number. If we could utilize this phenomenon, we can remove M. aeruginosa cells faster than expected. These mechanism could be considered in common with the reciprocity failure phenomena (Sommer et al. 1998; Grad & Williamson 2001). Yet, different behaviors of NIES-98 strain and PCC 7806 strain could not be explained well, it might be attributed to different metabolic activities even under same UV exposure condition. This point needs to be clarified further for a better understanding of UV inactivation mechanism of M. aeruginosa.

CONCLUSIONS

UV irradiated M. aeruginosa was incubated under white light (with photoreactivation) and yellow light (without photoreactivation). Effect of photoreactivation was studied and following conclusions were drawn.

(i) There was a large difference between photoreactivation condition and non photoreactivation condition; 2 log inactivation was achieved by 180 mJ cm$^{-2}$ of UV with photoreactivation, while only 30 mJ cm$^{-2}$ without photoreactivation.

(ii) Over 180 mJ cm$^{-2}$, UV inactivation mechanism could be attributed to other than DNA damage.

(iii) UV inactivation efficiency can be improved by inhibiting photoreactivation.

REFERENCES


